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Introduction

It is estimated that one in seven couples will experience fertility issues throughout their reproductive lives. The male factor, which is the single most common cause of infertility, is solely responsible in 30 % and contributory in an additional 30 % of cases [1, 2]. Assisted reproductive technologies (ART) are often considered as the first-line treatment to achieve pregnancy in infertile couples.

ART bypasses seminal abnormalities, such as a reduced sperm count, motility and percentage of morphologically normal cells. Traditionally, the evaluation of male fertility potential has relied upon microscopic assessment to determine semen quality [3]. Evaluation of sperm morphology has been known as the best prognostic indicator of spontaneous pregnancies [4], intra-uterine insemination [5], and conventional IVF [6] success.

However, the standard morphology evaluation on random stained cells from the ejaculate is of

limited value during intracytoplasmic sperm injection (ICSI). Because the ICSI procedure involves the direct injection of the spermatozoon into the oocyte, embryologists considered that the morphological evaluation of male gametes was of secondary importance [7].

It is now well established that the spermatozoon is not only a genetic material carrier to the oocyte. The human spermatozoon is crucial for contributing three components: (1) the paternal genome, (2) the signal to initiate oocyte activation, and (3) the centriole, which participates in the initial zygote development [8]. Therefore, ICSI has created concerns over the possibility of a paternal influence because the fertilizing spermatozoon have highly dynamic and essential participation in embryogenesis that and may be determinant of compromised embryo development [8–12].

A spermatozoon considered as morphologically normal under a magnification of 400× (Fig. 15.1) may carry minor morphological defects that impair the fertilization process and embryonic development. In the past decade a new approach involving real-time, high-magnification (up to 6,600×) observation of unstained spermatozoa, named "motile sperm organelle morphology examination" (MSOME), has been introduced [13] (Fig. 15.2). MSOME is able to identify mainly sperm head vacuoles, considered as nuclear defects (Fig. 15.3) [13] that may be associated with DNA and chromosomal abnormalities.

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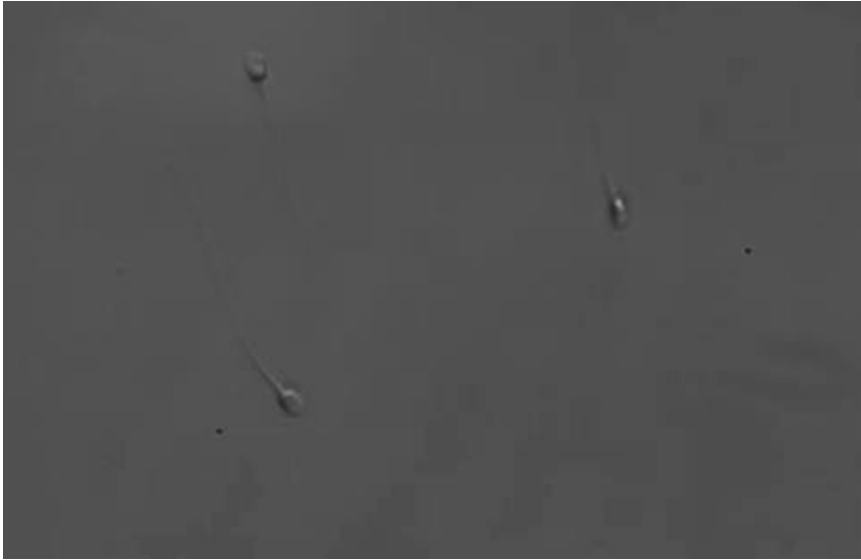


Fig. 15.1 Spermatozoa visualized under a magnification of 400×



Fig. 15.2 Spermatozoa visualized by MSOME (6,600×)

Because all the available tests for functional and genetic sperm assessment are extremely cytotoxic, sperm DNA integrity, and chromosomal constitution cannot be assessed in the sperm cell used for ICSI.

Sperm Chromosomal Constitution

In males, meiosis begins with puberty and occurs continuously throughout adulthood. Through meiosis I (MI), primary spermatocytes divide into



Fig. 15.3 Sperm with a large nuclear vacuole under MSOME (6,600×)

The human spermatozoon is an haploid cell ($n=23$) that contains 22 autosomes and one sex chromosome, either the X or Y. Anomalies in the sperm genetic information are known as numerical and structural chromosomal abnormalities. Numerical abnormalities comprise aneuploidies and polyploidies and structural abnormalities include chromosome breaks, gaps, inversions, insertions, deletions, translocations, and acentric fragments [14].

Techniques for Sperm Chromosomal Analysis

two secondary spermatocytes, and through meiosis II (MII) each secondary spermatocyte divides into two spermatids, which will differentiate and mature into a spermatozoon (Fig. 15.4).

Tremendous progress has been made studying the cytogenetics of male gamete. In 1970, the first chromosomal studies of spermatozoa were developed, using the differential staining of

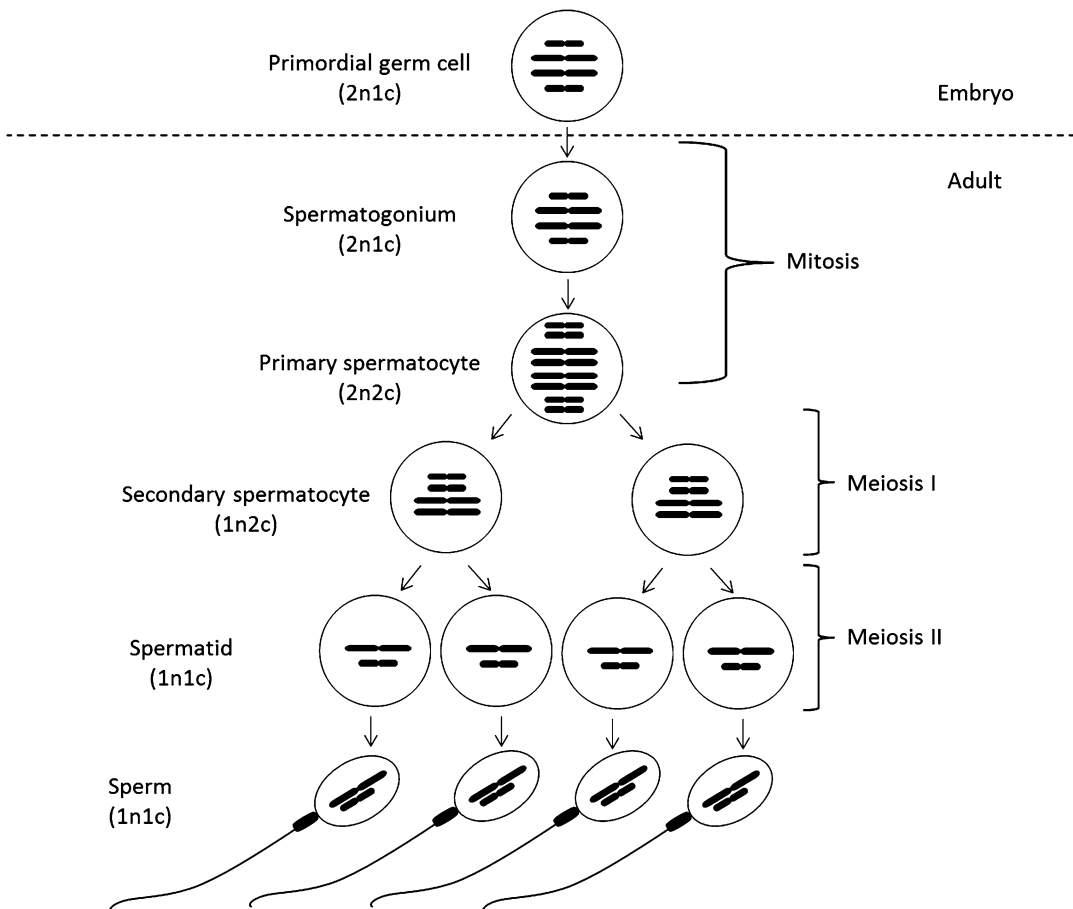


Fig. 15.4 Gamete formation in males (spermatogenesis)

specific regions of chromosomes [15]. An average aneuploidy rate of ~2 % per chromosome was reported, giving a total aneuploidy rate of 38 % if all chromosomes were considered together [16]. However, due to nonspecific staining of chromosomes, these estimates were considered excessive and untrustworthy. Thus, a more reliable technique was necessary.

In 1978, Rudak et al. [17] settled a system in which human sperm were introduced to hamster eggs which then proceeded through the initial stages of development. After that, cells were fixed and stained using karyotyping methods to observe metaphase nuclei, creating the first extended cytogenetic observation of human chromosomes, in which numerical as well as structural abnormalities could be analyzed.

In the 1990s, the first fluorescent in situ hybridization (FISH) assay was developed, offering a rapid, accurate and reliable technique for the identification of aneuploidy and polyploidy in human sperm [18]. However, due to the small size of the sperm head, FISH cannot be performed for all chromosomes because signals would overlap. Therefore, FISH is habitually performed for chromosomes related to aneuploidies that can result in live birth (chromosomes 13, 18, 21, X, and Y) [19].

Male Infertility and Chromosome Abnormalities

Human male infertility and chromosome abnormalities are frequently closely related. It has been reported that, in sperm of fertile men, the frequency of numerical and structural chromosome abnormalities varies from 1 to 2 % and 7 to 14 %, respectively [14]. Infertile patients have an increased incidence of chromosomal abnormalities [20], being the most common the aneuploidies, Y chromosome structural abnormalities, Robertsonian and reciprocal translocations, and chromosome inversions [21].

Alterations of semen parameters, including oligozoospermia, asthenozoospermia, and teratozoospermia, appear to be associated with increased sperm aneuploidy. Oligozoospermia

was proven to be related to sperm chromosomal alterations [22, 23], but the highest levels are reported in men affected by severe oligoasthenoteratozoospermia and in men suffering from non-obstructive azoospermia [24, 25].

Despite aneuploid spermatozoa are still able of fertilizing eggs, their use for ICSI is associated with reduced pregnancy rates, recurrent abortion, and chromosomal aberrations in the offspring [26, 27].

MSOME and Chromosomal Abnormalities

Sperm chromosomal constitution cannot be assessed in the sperm cell used for ICSI, therefore, several studies have investigated the relationship between sperm morphology by MSOME and sperm chromosomal status.

Garolla et al. [28] evaluated the mitochondrial status, DNA integrity, DNA fragmentation, and sperm aneuploidies in normozoospermic subjects and in two groups of patients with primary testicular damage or partial obstruction of the seminal tract. Moreover, in patients with severe testicular impairment, mitochondria, DNA, and chromosomes were reanalyzed on a single spermatozoon, selected at a magnification of 13,000× on the basis of normal morphology. Patients with testicular damage showed increased sperm aneuploidies as compared to the controls. In contrast, in the PO group the mean percentage of sperm aneuploidies was not different from controls. From semen samples of the ten patients with testicular damage, a total of 20 single immotile sperm cells per patient were retrieved and classified on the basis of normal morphology and absence or presence of vacuoles. FISH analysis in these cells showed that no chromosomal alteration was present in morphologically normal sperm cells. It is important to highlight that in this study a different equipment setting, able to multiply the sperm image up to 13,000×, was adopted. The authors showed that no matter the initial status of the whole sperm sample, spermatozoa selected by this method have lower incidence of DNA and chromosome alterations and

concluded that, especially in patients with severe testicular damage, the amplified use of MSOME could increase the efficacy and safety of the ICSI procedure, thus improving the outcome of male factor infertility treatment, above all in patients with severe testicular damage.

de Almeida Ferreira Braga et al. [29] investigated whether there was a connection between morphologic sperm normalcy evaluated through high magnification and sperm DNA integrity and sperm aneuploidy. The authors performed MSOME and FISH techniques in 200 sperm cells from 50 patients undergoing ICSI as a result of male infertility. The results showed that despite the presence of vacuoles and abnormal nuclear cell size was positively correlated with sperm DNA fragmentation, there was no correlation between these morphological features and aneuploidy. This result is in disagreement with the findings of Garolla et al. [28] and the authors justified that the studies' designs were very different, since de Almeida Ferreira Braga et al. analyzed the incidence of sperm aneuploidy in 200 cells, and Garolla et al. evaluated a single cell under high magnification and analyzed for sperm aneuploidies, which could explain the differences found between the studies.

Perdrix et al. [30] evaluated evaluate acrosome morphology, chromatin condensation, DNA fragmentation and sperm aneuploidy in spermatozoa with vacuoles occupying >13.0 % of sperm head area. For each of the 15 patients included in the analysis, results were compared with those obtained in spermatozoa from native semen sample. Results showed that aneuploidy and diploidy rates were significantly increased in sperm with large vacuoles. Nevertheless, due the low number of analyzed subjects, these results, as the authors themselves noted, should be interpreted with caution.

Watanabe et al. [31] utilized a human sperm chromosome assay to investigate whether the sperm vacuoles are related to DNA damage. Morphologically normal sperm (selected under 400× magnification) obtained from 17 patients and 3 fertile donors were analyzed for the presence of vacuoles under a magnification of 1,000×. In three patients and two donor samples, structural

chromosomal damage was evaluated in normal sperm containing large vacuoles. The frequency of chromosomal abnormalities in sperm selected under high-magnification was not significantly different from that obtained for sperm examined under 400× magnification. Nevertheless, it is important to note that the incidence of normal-shaped sperm with large vacuoles was sporadic and therefore chromosome analysis dealt with low numbers. Since the incidence of chromosomal abnormalities was twofold higher in vacuolated sperm than the value in normal-shaped sperm without vacuoles obtained from the same patients, one might argue that that difference could reach statistical significance in the analysis of a larger number of patients. In addition, it is worth mentioning that in this study sperm morphology was examined under a magnification of 1,000× while in the majority of studies a magnification of at least 6,000× was applied.

Boitrelle et al. [32] performed high-magnification morphological evaluation (10,000×), in 15 infertile patients, to select 450 morphologically normal spermatozoa and 450 spermatozoa with a large vacuole (occupying ≥ 25 % of the head area). Subsequently, chromatin condensation, DNA fragmentation and the status of chromosomes X, Y, and 18 in these spermatozoa were analyzed. The results showed that despite the presence of a vacuole was associated with impaired chromatin condensation, normal and vacuolated spermatozoa did not differ significantly in terms of aneuploidy.

It has been proven that in patients with macrocephalic sperm head syndrome normal-head spermatozoa can be retrieved but these spermatozoa are often aneuploid [33]. Chelli et al. [34] investigated two infertile males with macrocephalic sperm head syndrome originated from North Africa. Norma-headed spermatozoa were selected under 400× and 1,000× magnification and the FISH analysis was performed on those selected spermatozoa. A total of 39 spermatozoa were selected under 400× and 6 were selected under 1,000×. A statistically significant decrease in diploidy and an increase in haploidy were observed in MSOME-selected spermatozoa as compared to sperm selected under 1,000×.

Despite the selection by MSOME resulted in significant elimination of sperm polyploidy and diploidy it did not eliminate the select of aneuploid spermatozoa. The authors highlighted that their results should be viewed with caution because only six spermatozoa were retrieved. Nevertheless, the absence of vacuoles after MSOME analysis was not a guarantee of normal chromosome content in these patients.

Another study evaluated whether high-magnification observation of spermatozoa in translocation carriers is related to sperm morphology and chromosomal content. Nine men carrying either a balanced reciprocal or a Robertsonian translocation were included in the study. The results showed that the absence of sperm vacuoles by MSOME was not sufficient to avoid spermatozoa with an unbalanced chromosomal content in patients carrying a reciprocal or a Robertsonian translocation [35].

Individual chromosomes reside in distinct territories [36, 37] and the preferential longitudinal positioning has been recognized for 11 chromosomes in human sperm [38]. Chromosomes X, Y, and 18 positioning has also been compared between spermatozoa with large vacuoles and normal spermatozoa analyzed and the results showed that chromosome architecture was modified in spermatozoa with large vacuoles compared with normal spermatozoa [39].

Conclusion

Recently, the MSOME, a noninvasive technique of sperm selection has been proposed to best predict ICSI outcome. The MSOME allows the selection of sperm cells with better physiological status and has been reported to result in improved implantation and pregnancy rates and reduced miscarriage rates. Few studies have investigated the chromosomal contents of morphologically normal and abnormal sperm cells selected by MSOME; however, to date, the results are still controversial. These discrepancies may be explained by (1) the lack of definition regarding the size of a large nuclear vacuole, (2) the difference in the total calculated magnification applied

in sperm analysis, and (3) the characteristics of the patients analyzed in each study. Therefore, further studies are necessary to determine whether or not the presence of sperm vacuoles correlates with sperm chromosomal status.

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